Biological Activity of Some Monocyclic- and Bicyclic β-Lactams with Specified Functional Groups

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Abstract: This Review contains our recent studies on evaluation of biological activities associated with monocyclic β-lactams and bicyclic β-lactam antibiotics containing various heteroatoms. A series of bicyclic β-lactams was synthesized, which possessed electron-withdrawing groups, such as an ester, mesylate, and triflate functionality. These β-lactams exhibited enhanced antibacterial activity.

1. INTRODUCTION

β-Lactam antibiotics act as potent antibacterial agents with a very low incidence of side effects. The naturally occurring β-lactam antibiotics, such as penicillin **1** and cephalosporin **2**, are originally discovered in fungi and later detected in *streptomycetes* [1]. These compounds possess a β-lactam ring and are of biological importance [1,2]. Often β-lactams can inhibit the transpeptidase, which is responsible for cross-linking of the peptidoglycan chains used in the bacterial cell wall synthesis [1-6].

Nocardicins **3**, the monocyclic azetidinones, are the first naturally occurring monocyclic β-lactams discovered [7]. These β-lactams are structurally and biologically related to penicillins and cephalosporins, which possess significant antibacterial activity [7]. Nocardicins are more active against *gram*-negative than *gram*-positive microorganisms *in vivo*. Considerable existing evidence indicates that their action differs from that of the classical β-lactam antibiotics [8]

The unique structural and chemotherapeutic properties exhibited by β-lactam antibiotics attract much attention of medicinal chemists to design and study new therapeutic agents with extended biological activity. In this short Review, we forcus our discussion on evaluation of biological activity resulting from monocyclic β-lactams, electronically activated isodethiaoxacephems, isocephems, isodethiaselenacephems, isodethiaazacephems, as well as isodethiaazacepham. Biological activities (e.g., the minimum inhibitory concentration) exhibited by these compounds, however, may not be related only to the functional groups present therein. Their minimum inhibitory concentrations are also dependent on other properties, such as compound stability, bacterial cell-wall permeability, etc.

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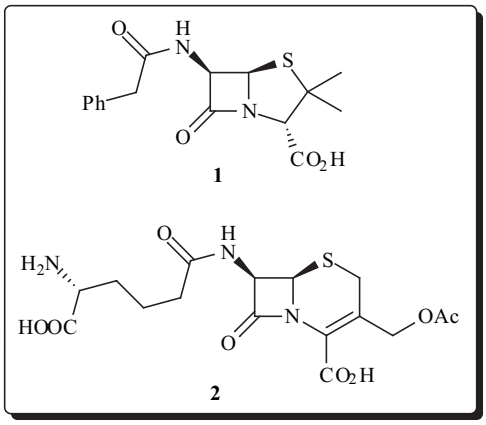

2. MONOCYCLIC β-LACTAMS AND MASKED *p*-QUINONE METHIDE β-LACTAM AS AN ACTIVE METABOLITE OF NOCARDICINS

This section covers discussions of biological activities associated with nuclear analogs of nocardicin A (**3**) and electronically activated monocyclic β-lactams. Introduction of ring strain or electronic activation onto monocyclic β-lactams did not lead to greater potency. Nevertheless, presence of a phenolic hydroxyl group was found essential for their significant antibacterial activity. For the biological activities exhibited by nocardicins, a mechanism was proposed that involved oxidation of the phenolic hydroxyl moiety to the corresponding quinone methide metabolites.

2.1 Effect of Ring Strain in Nocardicins and Synthesis of Masked *p*-Quinone Methide β-Lactam

The β-lactam ring in nocardicins A (**3**) is relatively unstrained in comparison with classical β-lactam antibiotics. It is stable towards nucleophilic attack [9]. When additional ring strain is placed on the β-lactam ring, the resultant analogs (e.g., **4** and **5**) do not exhibit greater potency nor a broader spectrum of antibacterial activities [10,11].

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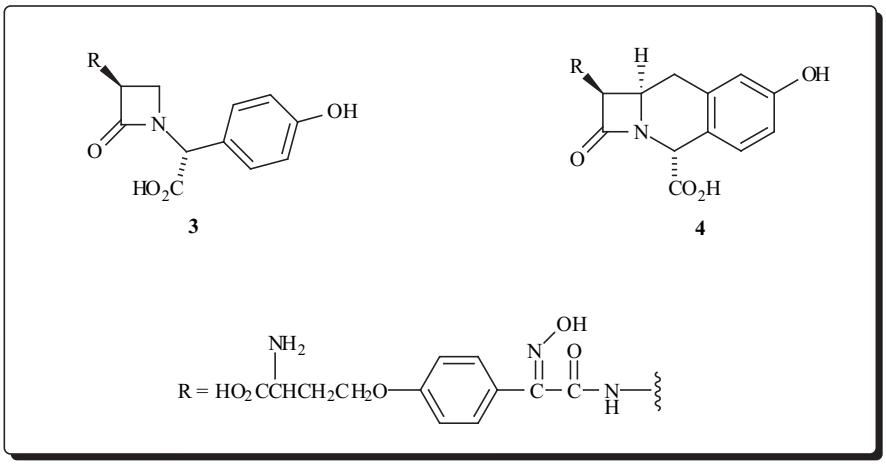
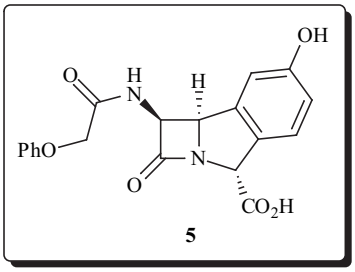


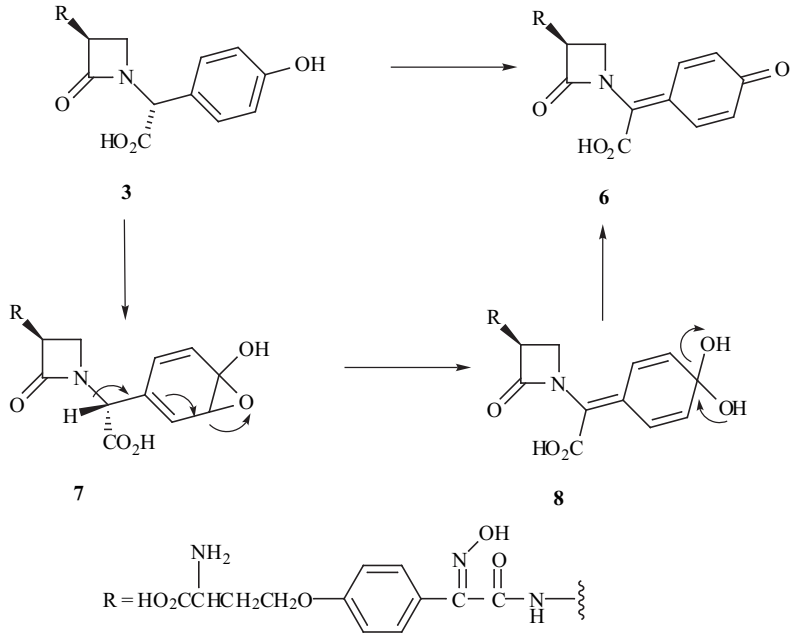
In 1979, Boucherot and Pilgrim [12] reported the generic quinone methide structure of **6**. The conjugated quinoid was prepared as the active species by analogy with the electronic structure of the active conjugated 3-cephem, and in contrast to that of the inactive non-conjugated 2-cephem. The non-classical β-lactams in the series of compounds **4** and **5** could be readily recognized and oxidized by an oxidative enzyme *in vivo* to give the corresponding quinone methide metabolites **6** (Scheme 1) [8]. These metabolites may inhibit



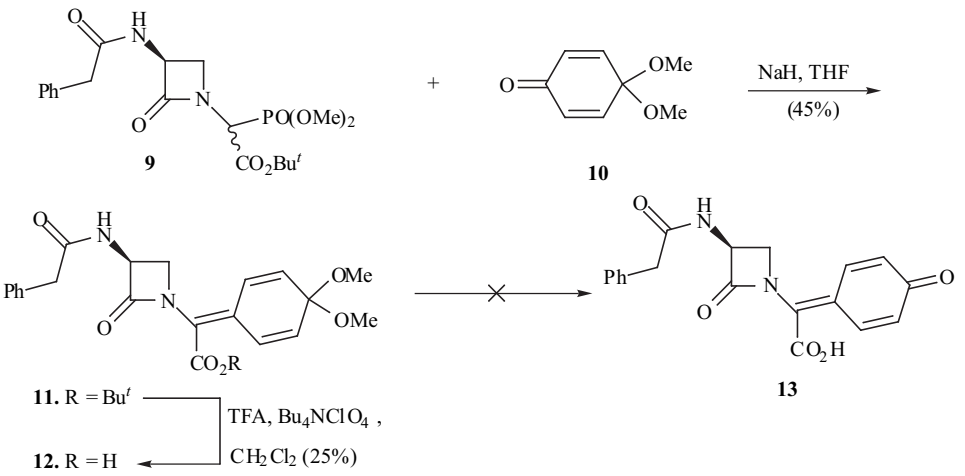
cell wall synthesis of bacteria. An alternative mechanism was also proposed for their mode of action in biological systems. It involved the epoxidation of phenolic moiety of nocardicins *in vivo* followed by their conversion to the corresponding cyclohexadienylidenes **8** [8]

The synthetic strategy used for the preparation of *p*-quinone methide β-lactam derivative **13** is illustrated in Scheme 2 [8]. Monocyclic β-lactam **9** reacted with 4,4-dimethoxycyclohexa-2,5-dien-1-one (**10**) in the presence of NaH in THF to produce the corresponding masked *p*-quinone methide **11**. Hydrolysis of *tert*-butyl ester **11** with CF_3CO_2H and a trace amount of Bu_4NClO_4 in CH_2Cl_2 produced the carboxylic acid **12** in 25% overall yield.

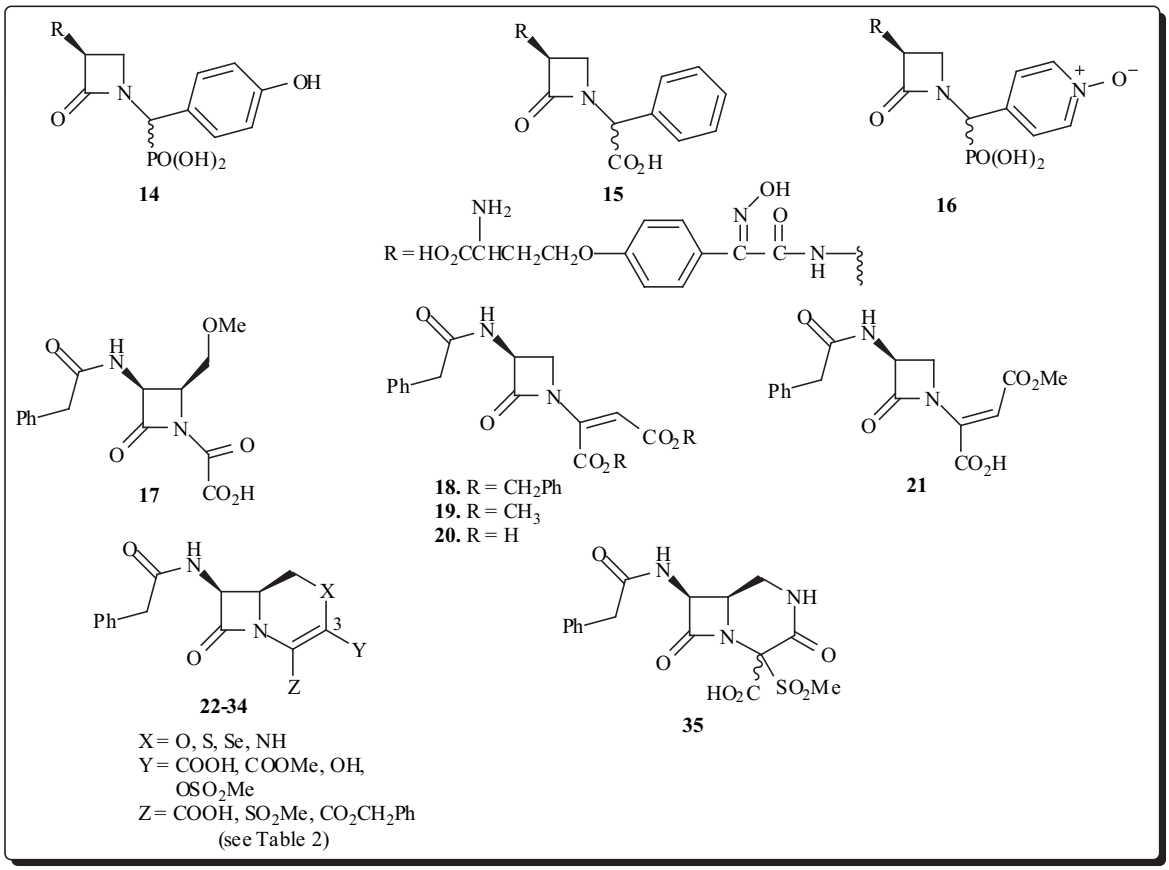
All attempts to convert **12** to the quinone methide **13** resulted in the destruction of the β-lactam ring [8]. Nevertheless, carboxylic acid **12** which was the precursor of *p*-quinone methide β-lactam **13** exhibited significant biological activity (see Table 1). A plausible mode of action [13] may require the phenolic group to be present initially in the molecule for its membrane penetration. Thus it provides assisted access to the target. An oxidative cascade involving a quinone acetal followed by acylation of the target would be a logical sequence of events. Target acylation by a species,



Scheme 1.



Scheme 2.



18. R = CH_2Ph
19. R = CH_3
20. R = H

such as **13**, affords a possible mechanism involving ring opening of the β-lactam with concomitant aromatization of the quinoid ring—an energetically favorable process.

2.2 Significance of Phenolic Hydroxyl Group of Nocardicin A (3)

The importance of the phenolic hydroxyl group of nocardicin A (**3**) has been investigated [8]. Its phosphonate derivative **14** was synthesized and a comparative study of the biological activities of **3** and **14** was made with the dehydroxylated nocardicin **15** and pyridinium *N*-oxide analog **16**. Nocardicins **14**–**16** and *p*-quinone methide β-lactam derivative **12** were tested *in vitro* against five pathogenic microorganisms up to a level of 800 μg/mL [8]. The results in Table 1 show that the dehydroxy derivative **15** did not exhibit any biological activity. These results are in contrast with the notable antimicrobial property of nocardicin A (**3**) and its phosphonate derivative **14**. Thus the phenolic OH group must play an important role on the biological activity.

Pronounced antimicrobial effect resulting from β-lactam **12** is in accordance with our proposed mechanism that involves the oxidation of the phenolic moiety in nocardicins to the corresponding quinone methide metabolites (Scheme 1) [8]. This process could be responsible for the notable

Table 1. Antibacterial Activity of β-Lactams 3, 12, and 14–16 against Pathogenic Microorganisms[a)]

β-lactam	*S. aureus* FDA-209P	*S. lutea* PCI-1001	*P. vulgaris* IAM-1025	*P. mirabilis* 1432-75	*P. aeruginosa* 1101-75
3	800	6.25	1.56–3.13	1.56	12.50
12	176.80	4.65	1.87	0.86	6.25
14	>800	38.56	21.34	15.63	18.79
15	>800	>800	>800	>800	>800
16	48.75	>800	>800	>800	>800

a) Minimum Inhibitory Concentration [μg/mL]

antibacterial effect *in vivo*. It was further supported by the lack of activity of pyridinium *N*-oxide **16** against *gram*-negative microorganisms. Nevertheless, β-lactam **16** exhibited moderate activity against *gram*-positive *S. aureus* bacterium.

2.3 Electronically Activated Monocyclic β-Lactams

The monocyclic β-lactam analogs **17**–**21** are synthesized, in which the ring strain of the fused β-lactam is replaced by a functional group with electronic activation capability [14–16]. Just *et al.* [14] reported the preparation of β-lactam **17**, in which the electronic activation is provided by a carbonyl group attached to the β-lactam nitrogen. Although **17** absorbs at ν_{max} 1810 cm^{-1}, it is not active against *gram*-negative nor *gram*-positive organisms at a level as high as 128 μg/mL [14]. This indicates that although a high absorption frequency of β-lactam in IR is an essential prerequisite for great antimicrobial activity, it may not always lead to a compound with biological activity. Similarly, though the electronically activated β-lactam esters **18** and **19** absorb at higher IR frequency (ν_{max} 1790 cm^{-1}), the corresponding acid **20** showed no significant antibacterial activity in phosphate buffer at pH 7.0 [15].

Results from kinetic studies indicate that the dicarboxylic acid **20** is not readily attacked by nucleophiles. This low susceptibility to nucleophilic attack and lack of biological activity may be due to reduced electron-withdrawing ability of the maleate moiety in **20** by anion formation at neutral pH conditions [15]. The electronic activation of monocyclic β-lactams of type **20** is not sufficient to generate a biologically active compound.

Consequently, the β-lactam **21** was synthesized in which the electronic activation was provided by an ester functionality [17]. In the IR spectrum, β-lactam **21** absorbed at ν_{max} 1788 cm^{-1} and showed susceptibility to nucleophilic attack. Nevertheless, it was biologically inactive.

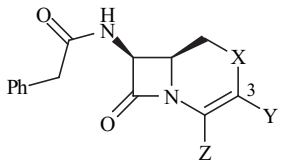
3. ELECTRONICALLY ACTIVATED BICYCLIC B-LACTAMS AS POTENT ANTIBACTERIAL AGENTS

In this section, syntheses and evaluation of antibacterial properties of electronically activated bicyclic β-lactams are presented, which contain an oxygen, sulfur, selenium, or nitrogen atom. In these compounds, electronic activation was induced by introduction of a carboxylic acid, ester, mesylate, or triflate functionality at the C-3 or the C-4 position in the bicyclic β-lactam ring. The presence of the triflate, mesylate, and ester groups enhanced the antibacterial activity of the bicyclic β-lactams.

3.1 Synthesis and Biological Activities of Electronically Activated Bicyclic β-Lactams

Being susceptible to nucleophilic attack, β-lactam **21** did not exhibit any antibacterial activity. It may be necessary for the enamine fragment in β-lactams to be prevented from being coplanar with the β-lactam nucleus [17,18]. As fused β-lactams met this requirement, heteroatom-containing

Table 2. Electronically Activated Bicyclic β-Lactams 22–34



β-lactam	X	Y	Z
22	O	COOEt	COOH
23	O	COOH	COOH
24	O	COOMe	COOH
25	S	COOH	COOH
26	S	COOMe	COOH
27	Se	COOH	COOH
28	Se	COOMe	COOH
29	NH	OH	COOH
30	NH	OH	SO_2Me
31	NH	OSO_2Me	COOH
32	NH	OSO_2Me	CO_2CH_2Ph
33	NH	OSO_2CF_3	COOH
34	NH	OSO_2CF_3	CO_2CH_2Ph

Table 3. Antibacterial Activity of Monocyclic β-Lactams 17 and 21 as well as Bicyclic β-Lactams 22–35 against Pathogenic Microorganisms[a)]

β-lactam	*S. aureus* FDA-209P	*E. coli* ATCC 39188	*S. typhi* 0-901	*P. aeruginosa* 1101-75	*K. pneumoniae* NCTC 418
17	>128	>128	>128	>128	>128
21	>128	>128	>128	>128	>128
22	0.02	0.37	2.50	15.00	0.27
23	0.83	16.56	23.94	55.89	19.47
24	0.03	0.56	3.94	15.89	0.47
25	0.85	13.00	26.00	50.00	20.00
26	0.07	0.65	1.50	13.00	2.15
27	1.20	15.35	38.65	39.45	25.60
28	0.10	1.25	2.05	8.95	3.54
29	29.50	94.68	>128	>128	>128
30	>128	>128	>128	>128	>128
31	0.07	0.95	1.20	4.38	0.68
32	>128	>128	>128	>128	>128
33	0.01	0.09	0.68	1.15	0.24
34	>128	>128	>128	>128	>128
35	48.50	97.17	65.30	120.00	51.20
Ampicillin	0.33	2.51	>128	>128	>128
Cloxacillin	0.18	1.70	>128	>128	>128
Penicillin G	0.40	2.30	>128	>128	>128

(a) Minimum Inhibitory Concentration [μg/mL]

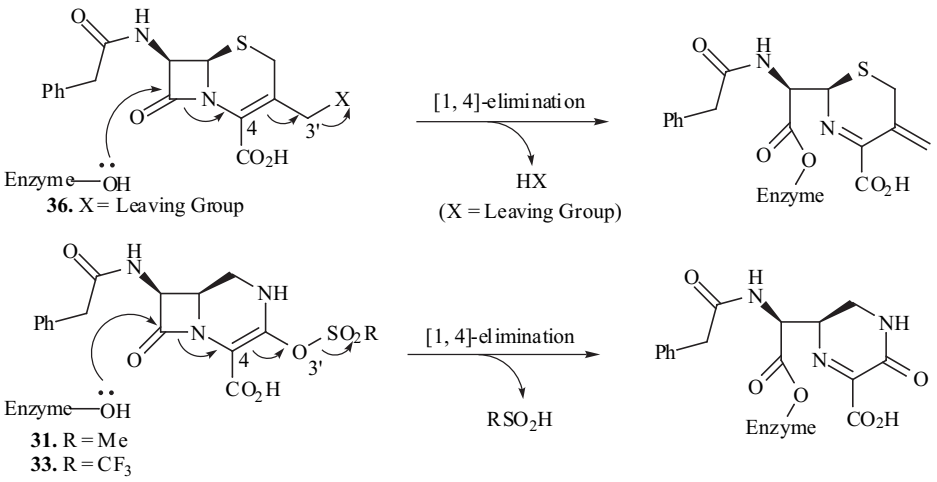
bicyclic β-lactam derivatives **22**–**35** (see Table 2) were synthesized by chemical methods [18–23].

Bicyclic β-lactams **22**–**35** and monocyclic β-lactams **17** and **21** were tested *in vitro* against five pathogenic microorganisms [19–22]. Ampicillin, cloxacillin, and penicillin G were used as reference compounds. The bicyclic β-lactams **22**–**34** shown in Table 3 were activated electronically by a C=C–COOR functionality. Freedom existed in monocyclic β-lactams **17** and **21**. In contrast, the π-electrons of the C–C double bond of the strained bicyclic β-lactams **22**–**34** cannot be aligned perfectly with the unshared pair of electrons of the nitrogen atom. This discrepancy could account for the difference of biological activity between monocyclic and bicyclic β-lactams [18]. All of the highly strained isooxacephems **22** and **24**, isocephem **26**, and isoselenacephem **28** possessed an ester functionality at the C-3 position. These compounds exhibited greater antimicrobial effect than the bicyclic β-lactams **25** and **27**, which possessed a carboxylic group at the C-3 position. Thus, the electronic activation of the β-lactam moiety by an ester functionality played an important role on the biological activity of bicyclic β-lactams [19–21]. Furthermore, isocephem **26** had an LD_{50} (*i.v.*) of ~800 μg/kg and isoselenacephem **28** had an LD_{50} (*i.v.*) of ~180 μg/kg in rats. Compounds **26** and **28** showed similar antimicrobial activity but exhibited different toxicity [21].

3.2 Significance of Leaving Groups at the C-3 and the C-4 Positions in Cephalosporins

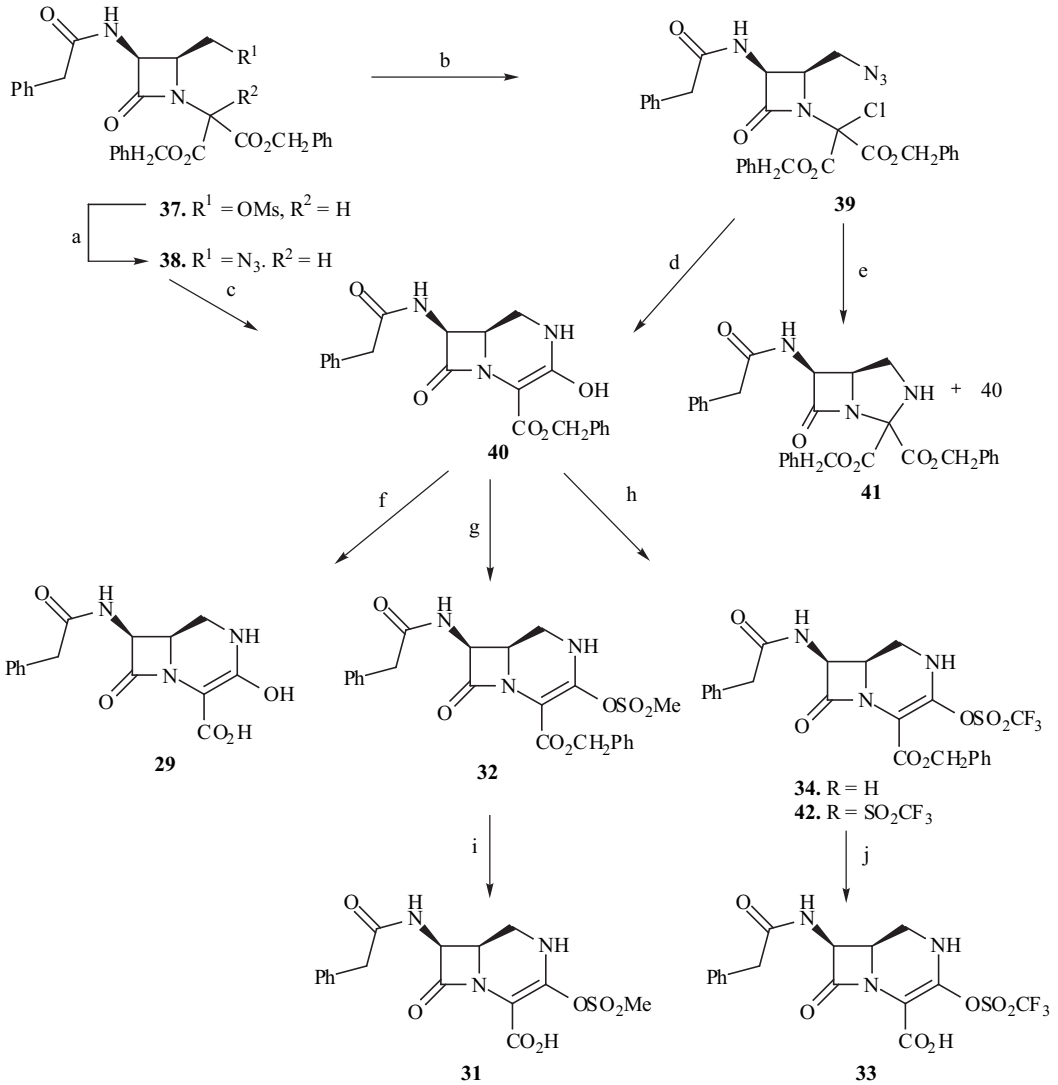
β-Lactam antibiotics exert certain biological activity by acylating serine residues of transpeptidases [22], so that the cross linking of peptidoglycans does not occur [22,24]. For the enhancement of their antibacterial activity, a leaving group may be attached to the C-3' position of cephalosporins (Scheme 3). When cephalosporin **36** reacts with enzymes responsible for the cell wall synthesis of bacteria, ring opening of the β-lactam nucleus would occur. As a result, liberation of a leaving group takes place through a 1,4-elimination process [25–29]. When the eliminated species possesses excellent leaving ability, cephalosporins may exhibit profound antibacterial activity [25,26,30–34].

Accordingly, a single β-lactam mesylate **37** was designed as the starting material in the synthesis of the desired bicyclic β-lactams **29**, **31**, and **33** (see Scheme 4) [23,35]. The intermediates **32**, **34**, **39**, **40**, and **42** were fully characterized by spectroscopic methods. On the other hand,



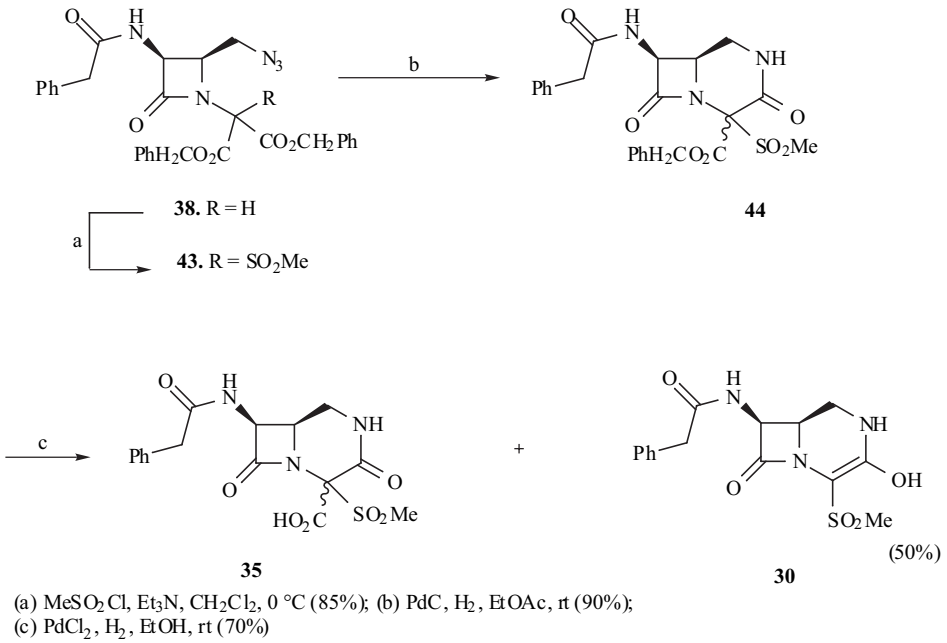
Scheme 3.

isodethiaazacepham **35** bearing a mesyl group at the C-4 position was synthesized from azido β-lactam **38** via bicyclic β-lactam **44** (Scheme 5) [23]. Decarboxylated β-lactam **30** was produced as a by-product.



(a) NaN_3, DMF, rt (90%); (b) CF_3SO_2Cl, Et_3N, CH_2Cl_2, 0 °C-rt (90%); (c) Pd/C, H_2, EtOH, rt (94%); (d) Pd/C, H_2, EtOAc, rt (87%); (e) H_2S, Et_3N, CH_2Cl_2, rt (55%); (f) $PdCl_2$, H_2, EtOH, rt (50%); (g) $MeSO_2Cl$, pyridine, CH_2Cl_2, 15 °C (45%); (h) CF_3SO_2Cl, pyridine, CH_2Cl_2, 15 °C (40%); (i) $PdCl_2$, H_2, EtOH, rt (35%); (j) $PdCl_2$, H_2, EtOH, rt (30%)

Scheme 4.



Scheme 5.

The biological results of bicyclic β-lactams **29**–**35** are listed in Table 3. Bicyclic β-lactams **31** and **33** possessed excellent antimicrobial properties [23]. In contrast, enol β-lactam **29** exhibited much lower activity. The sulfone moiety, acting as a leaving group, at the O-3' position of **31** and **33** enhanced their antibacterial activity. Trifluoromethane sulfone moiety in **33** possessed greater leaving capability than methanesulfone unit in **31**. Thus antibacterial activity was more potent for isodethiaazacephem **33** than **31** [23]. This is in agreement with the hypothesis on their mode of action in biological systems (see Scheme 3) [23]. These results indicate the biological importance of mesylate and triflate functionalities at the C-3 position of cephalosporins.

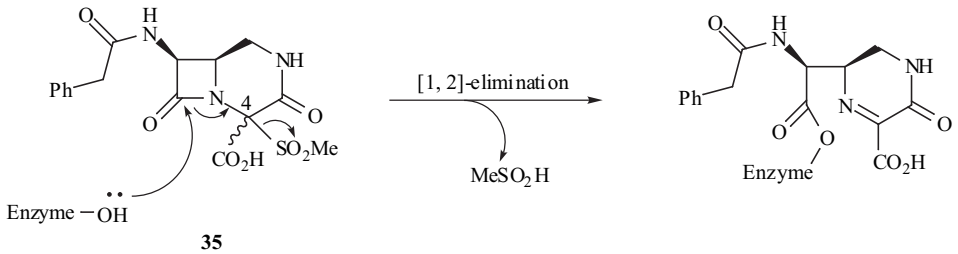
On the other hand, benzyl ester derivatives **32** and **34** failed to show antibacterial activity although both of these two compounds possess SO_2R as the leaving group. It showed that the chemical reactivity and the recognition capability of a substrate by the target enzymes are essential factors for effective biological activity [23].

Upon recognition of the feasibility of 1,4-elimination in β-lactam antibiotics (Scheme 3), the possibility of a 1,2-elimination process was considered to occur in isodethiaazacepham **35** [23]. It bears a mesyl moiety as the leaving group at the C-4 position and the elimination process could also be initiated by bacterial enzymes (Scheme 6). Nevertheless, cepham sulfone **35** was unstable and lack of significant biological activity (see Table 3). Under physiological conditions, decarboxylation of **35** took place to give a biologically inactive compound **30** (Scheme 7). The presence of a carboxyl group at the C-4 position of cephalosporins **35** was essential for recognition by the target enzymes, such as penicillin-binding proteins. Compound **30** was lack of such a functionality and thus cannot interact with the target enzymes. Under acidic conditions, destruction of the β-lactam moiety in cepham sulfone **35** occurred to give **45** [23]. These results indicate that presence of a good leaving group at the C-3 and the C-4 positions in cephalosporins enhanced their antibacterial activity.

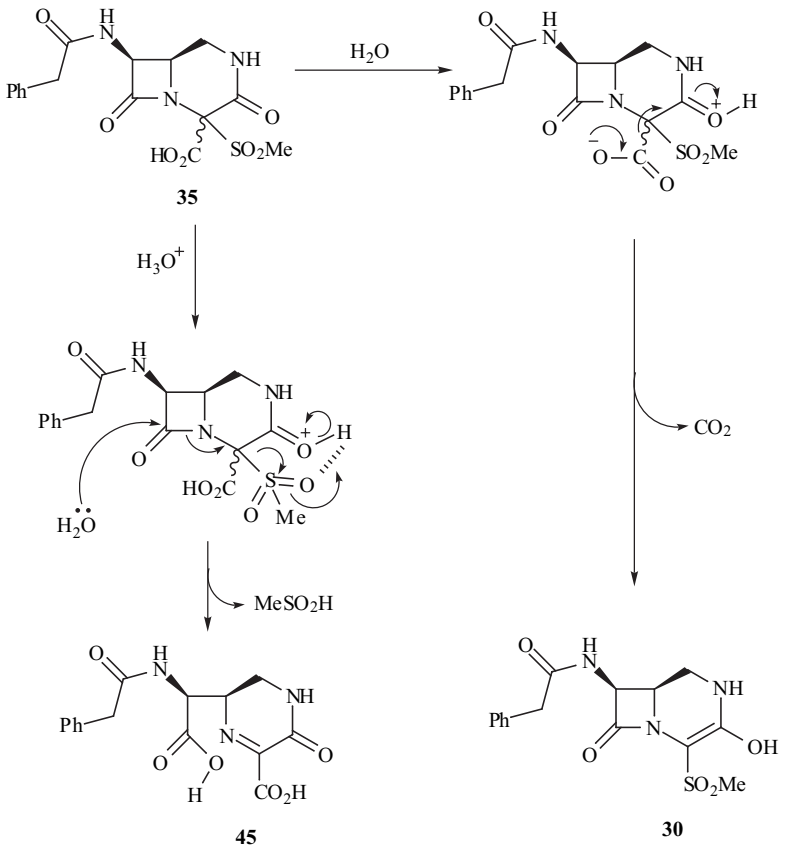
4. CONCLUSIONS

This Review summarizes our studies on biological activities of some monocyclic and bicyclic β-lactams. Monocyclic β-lactams, such as nocardicins, possessed remarkable antimicrobial activity. The presence of a phenolic hydroxyl group on nocardicins was of importance for their biological activity.

A series of bicyclic β-lactams containing heteroatoms were synthesized with an electronically activated



Scheme 6.



Scheme 7.

functionality at a designed position. These compounds showed enhanced biological activity. In cephalosporins, the electronic activation can be induced by introduction of a carboxylic acid, ester, mesylate, or triflate functionality at the C-3 position of the bicyclic β-lactams. When the eliminated species was a good leaving group, cephalosporins could exhibit excellent antimicrobial activity. The mesylate and triflate functionalities in isodethiaazacephems acted as good leaving groups and, consequently, these isodethiaazacephems exhibited enhanced biological activities.

5. ACKNOWLEDGEMENTS

For financial support, we thank Ministry of Education, the National Science Council of Republic of China, and Academia Sinica.

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